Accomplishments: 1996-present

1996: Development of Reporter Gene Assay for Marine Toxins

A new assay technology has been developed for algal toxins. Reporter gene assays have been established using the c-fos response element linked to the coding region for firefly luciferase and this approach has been published (Analytical Biochemistry). This method is very effective for measuring brevetoxins, PSP toxins and ciguatoxins. The method has a particularly high sensitivity for ciguatoxins and should permit a high capacity monitoring of the toxin in small (<1 g) finfish samples. *Contact: John Ramsdell*

Growth Regulation of Toxic Dinoflagellates

Studies on growth regulation in dinoflagellates have defined the molecular mechanism by which the dinoflagellate cell cycle is phased to the diel cycle. Phasing is accomplished by an inhibitory signal in response to blue light. The blue light receptor in dinoflagellate cells has not yet been identified, but the signaling pathway appears to be dependent on cAMP, a signaling molecule involved in transmitting blue light signals in higher plants. Cell cycle regulatory mechanisms in toxic dinoflagellates will yield useful probes to study the dynamics of harmful algal blooms. Additional investigations carried out this year addressed the role of marine biotoxins in regulating growth dynamics in the ciguatera dinoflagellate community. Ciguatera associated toxins have been identified to elicit allelopathic effects against other co-occurring dinoflagellate species. Results of these studies will provide insight into mechanisms initiating ciguateric reef conditions. *Contact: Fran Van Dolah*

1997: Growth Kinetics of Red Tide Algae in the Gulf of Mexico

Studies on growth regulation in dinoflagellates have focused this year on the role of endogenous cellular rhythms in the initiation, growth, maintenance, and decline of harmful algal blooms. Field studies using flow cytometry have confirmed that cell division in a naturally occurring bloom of the Florida red tide organism, *G. breve*, is phased into the the diel cycle. The cell division rate in the bloom population was 0.2 div./day, similar to division rates obtained in the laboratory. No correlation was found between vertical distribution and cell division. Identification of the biochemical mechanisms controlling cell growth will advance prediction of the dynamics of harmful algal blooms. *Contact: Fran Van Dolah*

Collaborative Testing of Receptor Assays for Marine Toxins

Receptor based assays for PSP, ASP, NSP, and CFP have been developed and laboratory validation completed in the past four years. These assays are now ready to be tested corroboratively in formal interlaboratory trails. The first of these trails, testing the assay for NSP in oysters, has been initiated as an AOAC Peer Verified Method trial, which will be completed in FY1998. *Contact: Fran Van Dolah*

1998: Growth Control of Harmful Algal Blooms

Research on the biochemical pathways that control growth of red tide algae provides a new means to understand the processes that initiate harmful algal blooms and to evaluate measures to control growth of harmful algae. These pathways are amenable to chemical and biological intervention, such as that applied to inhibit growth of terrestrial plants. Current research efforts focus on the Florida red tide dinoflagellate, *Karenia brevis* , and the ciguatera associated dinoflagellates. Diel phasing of the cell cycle has been characterized in both laboratory cultures and field populations of the Florida red tide dinoflagellate, and the light dependent cues that couple the cell cycle to the diel cycle have been identified. The molecular regulators of the cell cycle have been shown to be sensitive to inhibition by a drug developed to inhibit growth of cancer cells. Studies on allelochemical interactions within the ciguatera dinoflagellate assemblage have identified a novel growth inhibitory compound produced by *Prorocentrum lima* and active against other dinoflagellates. Liquid chromatography-mass spectrometry has determined that this compound is unrelated to okadaic acid, the biotoxin produced by *P. lima*. Structural characterization and mode of action of this compound are currently are currently being addressed with LC-MS and nmr. *Contact: Fran Van Dolah*

Biological Control Measures for Harmful Algal Blooms

Research on the interaction of bacteria and red tide algae has provided a new means to understand microbial processes leading to the termination of harmful algal blooms. Clones of algicidal bacteria have been isolated from both bloom and non-bloom waters in the Gulf of Mexico, and one strain from each water type is able to terminate the growth of red tide algae in laboratory cultures. The algicidal activity of one bacterium has been partially characterized, and shown to correspond to a dissolved component which exhibits a degree of taxonomic specificity in its effect on target species. Defining the role of algicidal bacteria in algal bloom termination provides a basis for new generation management efforts necessary to control harmful algal blooms.

Contact: Greg Doucette

Definitive Chemical Analysis of Brevetoxin in West Indian Manatees

Many of the unusual marine mammal mortality events attributed to harmful algal blooms remain circumstantial. The highly publicized mortality of the West Indian manatees on the west Florida shore lacked chemical confirmation of brevetoxin exposure. Methodologies to obtain ultra low limits of detection and quantification values for brevetoxin have been achieved with APSI-mass spectrometry. First time blubber has been successfully analyzed opening a new potential of life history studies in manatees and/or other marine mammals. The very low detection limits afforded by LCMS coupled to the resolving power of LC as a MS front end has allowed this otherwise very difficult matrix to be analyzed. Laboratory training on the use of this methodology was provided this summer as part of a veterinary internship program. *Contact: Peter Moeller*

1999: Characterization of Algicidal Bacteria Toxic to Red Tide Algae

Research on the interaction of bacteria and red tide algae has provided a new means to understand microbial processes leading to the termination of harmful algal blooms. The 16S rRNA gene for two algicidal bacteria has been sequenced. Preliminary analyses indicate one strain is a member of the flexibacter-cytophaga subgroup of the cytophaga/flexibacter/bacteroides (CFB) phylum within the domain Bacteria, while the other strain is a member of the gamma-proteobacteria. Fluorescently-labeled rRNA probes have been designed for both taxa and are being optimized for in situ hybridization. A high-throughput bioassay for guiding fractionation of extracellular bacterial metabolites based on algicidal activity was developed, and has facilitated the chromatographic separation of an algicidal fraction from bacterial culture filtrate. Defining the role of algicidal bacteria in algal bloom termination provides a basis for new generation management efforts necessary to control harmful algal blooms. *Contact: Greg Doucette*

Biomonitoring Brevetoxin Exposure in Mammals Using Blood Spot Cards

We have developed a method to monitor the exposure of mammals to brevetoxins. This sampling involves collecting whole blood, applying the blood to a ½ inch diameter circle on a specially prepared blood collection card and allowing it to dry. The blood spots are extracted in the laboratory and total brevetoxin activity quantified using high throughput receptor binding assay and specific brevetoxin congeners analyzed by liquid chromotragraphy-tandem mass spectrometry. Toxicokinetic characterization has been conduced with laboratory mice. Mice were treated with 180 ug/kg brevetoxin-3. Whole blood was collected at time points between 0.5 and 24 hours of brevetoxin exposure and 0.1 ml was spotted on filter paper cards. Brevetoxin activity as determined by receptor assay increased between 0.5 and 4.0 hours and was decreased, yet detectable 24 hours after brevetoxin exposure. Tandem mass spectrometry was used to provide confirmation of brevetoxin-3. The mass spectrometry results paralleled those of receptor assay for time points between 0.5 and 4.0 hours exposure. However, brevetoxin-3 was not detected at 24 hours suggesting metabolism to another biologically active form of the toxin. We anticipate that this approach will provide a method to biomonitor for brevetoxins in living marine resources, protected species, and humans and are evaluating this biomonitoring method for other marine toxins as well. *Contact: John Ramsdell*

Brevetoxins Induce Embryo Toxicity and Developmental Abnormalities

Brevetoxins are lipophilic polyether toxins with documented neurotoxic effects on adult animals. In this study, we extend last years study of ciguatoxin to quantify the adverse developmental effects of brevetoxins using an exposure paradigm that parallels the maternal-oocyte transfer of toxin. Medakafish (*Oryzias latipis*) embryos are exposed to brevetoxin six hours post fertilization by microinjection of a small quantity (2 nanoliters) of brevetoxin (or vehicle) reconstituted in a fish oil (triolein) droplet. The brevetoxin-containing droplet is placed adjacent to the larger oil droplet of the yolk sac. Embryos microinjected with doses of 0.8 ng/egg (ppm) and higher of brevetoxin-1 exhibit pronounced cardiovascular (tachycardia) and muscular (hyperkinesis) activity by embryonic day four. Prior to hatching, morphological abnormalities were commonly found in embryos at the following lowest adverse effect levels: 1.1 ppm- lateral curvature of the spinal column; 3.1 ppm- herniation of brain and meninges though defects in the skull; and 3.4 ppm malpositioned eye. Hatching abnormalities are also commonly observed at brevetoxin doses of 2.0 ppm and higher with head-first, as opposed to the normal tail-first hatching. The observation of developmental abnormalities following brevetoxin exposure identifies a new spectrum of adverse effects that may be expected to occur following exposure to red tide events.

Contact: John Ramsdell

2000: Characterization of Bacteria Algicidal to Harmful Algal Species

We are continuing to examine interactions between algicidal bacteria and harmful algal species as a means to understand the microbial processes influencing bloom population dynamics. Denaturing gradient gel electrophoresis and fluorescent in-situ hybridization with oligonucleotide probes are being used to assess changes in microbial communities associated with *G. breve* following the introduction of algicidal bacteria to algal cultures. Following inoculation into bacteria-containing *G. breve* cultures: 1.) algicidal bacteria numbers rapidly increase; 2.) the microbial assemblage changes, with some bacterial taxa disappearing and others appearing throughout the killing event; and, 3.) algicidal activity of introduced bacteria seems to vary according to the target alga's physiological status, with resistance to attack decreasing with declining algal growth rate. Our data suggest that algicidal bacteria may be able to fill a vacant niche (or displace another organism from its niche) within a *G. breve* culture. Use of these molecular approaches will aid in determining whether similar processes occur during a natural *G. breve* bloom. *Contact: Greg Doucette*

Algal Growth Regulation and Signalling

The objectives of ongoing algal growth regulation and cell signaling studies on dinoflagellates are to better understand cellular mechanisms regulating dinoflagellate blooms formation and to identify potential molecular targets for control measures. Studies carried out this year focussed on the Florida red tide dinoflagellate, *G. breve*. In a continuation of cell cycle regulation studies, this year we have identified the presence of cyclin in *G. breve* using western blotting techniques and immunocytochemistry. This, in conjunction with our previous work on cyclin dependent kinase, defines the fundamental cell cycle regulatory apparatus in *G. breve*. A cDNA library has been made and is being screened for the cyclin gene(s). We have completed studies that identify a eukaryotic type cyclic AMP signaling pathway in dinoflagellates. A cyclic AMP dependent protein kinase in *A. operculatum* has been identified and its subunit structure characterized biochemically. Studies were initiated this year on stress proteins in *G. breve*. We have identified the presence of the chloroplast small heat shock protein (hsp) and hsp60. These studies will be continued in the next FY to identify their involvement in bloom termination and light, salinity, and temperature stress. *Contact: Fran VanDolah*

Assay Validation and Technology Transfer

As part of the U.N. sponsored technology transfer program on red tides in SE Asia, we conducted a training workshop on receptor assays in Manila, Philippines in December 1999. The workshop was attended by 14 particiants from 7 SE Asian countries. In addition, this year we hosted two individuals associated with this program for extensive receptor assay training in the laboratory: Ms. Cecilia Conaco, of the University of the Philippines (October-Nov 1999) and Ms. Mei Mei Ch'ng, of University of Malaysia (March – August 2000). We will host up to 3 additional personnel from participating nations during FY 2001. The program will then carry out a round robin interlaboratory comparison trial between participating nations in 2002. A receptor assay training workshop was held in May at CCEHBR to transfer this technology to representatives of two state regulatory agencies interested in its potential as a replacement for the mouse bioassay: California Dept. of Health and Florida DNR. *Contact: Fran Van Dolah*

Identification Of Brevetoxin In Bottlenose Dolphins Following The Gulf Of Mexico Mortality Event

Using brevetoxin (PbTx) receptor assays as a rapid screen and HPLC-MS/MS for chemical confirmation, we were able to demonstrate the presence of PbTx in livers of dolphins that died in the course of a *G. breve* bloom in the Florida panhandle region. From August 1999 until February 2000, over 120 bottlenose dolphins stranded in the Florida panhandle. This number represents more than a 4 fold increase from historic annual averages of bottlenose dolphin stranding for this area. In August - October, the strandings were principally localized in the Appalachicola area, concurrent with a bloom of *G. breve*. No strandings occurred in November. However, strandings again increased during December through February 2000 and were localized in the western counties, primarily in the Choctawhatchee Bay area, where a *G. breve* bloom persisted. Brevetoxin was previously implicated in the 1996 manatee epizootic. Levels of PbTx found in dolphin livers were similar to those reported in manatees in 1996. *Contact: Tod Leighfield*

2001: CDNA Library for Studies on Functional Genomics od Florida Red Tide Dinoflagellate, *Karenia brevis*

Understanding mechanisms controlling cell proliferation and toxicity of dinoflagellates is critical to the development of predictive indicators for the physiological status of blooms and identification of potential targets for species-specific control measures. Progress in this field is hampered by the overwhelming lack of knowledge of the genetic make-up of dinoflagellates: currently there are less than 50 expressed gene sequences for dinoflagellates in the NIH GenBank Database. Sequencing of a cDNA library, developed for the Florida red tide dinoflagellate, *Karenia brevis* (formerly *Gymnodinium breve*), was undertaken this year to help fill this void. Expressed sequence tags (EST) will be deposited in the dbEST database of GenBank and will be used for developing microarrays for assessing dinoflagellate gene expression. *Contact: Fran Van Dolah*

Microbial Communities can Protect HAB Species Against Algae-Killing Bacterial

As part of an investigation into potential strategies for mitigating the negative consequences of HABs, we are studying the role of bacteria as natural growth regulators of HAB species. We have discovered two algicidal (algae killing) bacteria targeting the Florida red tide dinoflagellate, *Karenia brevis*. These bacteria are able to rapidly lyse most *K. brevis* cultures, yet it appears that certain strains are resistant to algicidal attack. Recent experiments have demonstrated that this apparent resistance is due to the inhibition of algicidal bacteria growth by the microbial community associated with resistant *K. brevis* strains. Remarkably, both resistance as well as susceptibility to algicidal activity can be transferred between *K. brevis* cultures with the exchange of their respective unattached bacterial assemblages. Thus, it is clear if algicidal bacteria play a significant role in regulating HAB dynamics and are to be considered as a part of a possible control strategy, interactions with the ambient microbial community will play a crucial role in determining the effect of such bacteria on algal growth. *Contact: Greg Doucette*

Initiation of the South Carolina Phytoplankton Monitoring Network

The inaugural year for South Carolina Phytoplankton Monitoring Network began with great enthusiasm and the opening of a new home page http://www.chbr.noaa.gov/CoastalResearch/SCPMN/SCPMNmain.htm. This community outreach p rogram consists of high school marine science and biology classes monitoring local waters for the presence of possible harmful algal species. Teachers participating in the network attended a workshop on algal identification and sampling techniques. Currently, 12 teachers and approximately 170 students are actively sampling local waters for harmful algae. Based on the observations of these groups, a number of potentially harmful species have been detected in South Carolina, some for the first time. These include representatives of the genera *Prorocentrum*, *Pseudo-nitzschia*, *Heterosigma*, and *Akashiwo*. Additional community groups will be added to the network during the next year to extend coverage of this program along the coast of South Carolina. *Contact: Steve Morton*

Brevetoxin-2 Production and Purification for Metabolism/Detection Research

The lack of toxin standards for use in research has always been a problem for the health community. Without well-characterized and highly purified toxin standards or reference materials, it is difficult or impossible to probe a toxin's mode of action, metabolism, or natural degradation. Such data are needed to support research aimed at mitigating toxin exposures and toxic effects. During this past fiscal year, we have developed a chromatographic methodology to produce PbTx2 as determined by MS and NMR, and have provided four milligrams of highly purified PbTx2 to NOS as well as our FDA and EPA partners. With purified toxin in hand, NOS and its partners can now label this toxin with radioactive tritium (3H) and/or a stable isotope, deuterium (2H), for use in monitoring toxin metabolism and degradation within living systems. The deuterated toxin will also be used by NOS and its partners to develop accurate, highly sensitive detection methodologies using mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR). *Contact: Peter Moeller*

Toxicogenomics: A Global Approach to Assessing Marine Toxin Exposure and Effects

Toxin exposure almost always causes changes in gene expression, either directly, due to the specific interaction of a toxic agent with its receptor, or indirectly due to the induction of intracellular signaling cascades. Toxicogenomics is the application of DNA arrays to identify a specific pattern of gene expression induced by a particular toxicant. Once a "signature" gene response is identified, this information may be useful for elucidating a toxic mode of action and may potentially yield biomarkers of exposure unique for a particular toxicant or class of toxicants. This year the Marine Biotoxins Program co-organized a workshop on "Toxicogenomics and Nanotechnologies: New Frontiers for Mycotoxins and Phycotoxins" (June 22-23, 2001; Tufts University Bedford, MA) and carried out preliminary studies to determine the suitability of this approach for algal toxin exposure. Changes in gene expression in brains and livers of mice exposed to brevetoxin were studied. Several genes were found to be induced in response to this toxin class. Ongoing studies will determine the dose/response and time course of genetic responses and compare gene induction "signatures" of different algal toxin classes. *Contact: Fran Van Dolah*

2002: cDNA Library Provides Molecular Tools to Understand HAB Formation

Understanding the mechanisms that control the growth and toxicity of dinoflagellates has long been hampered by our lack of insight into their molecular biology, stemming from the lack of molecular tools needed for such investigations. Development and screening of a cDNA library containing expressed gene sequences from the Florida red tide dinoflagellate, *Karenia brevis,* was therefore initiated this year to provide some of these tools. This project has yielded novel insights into the intracellular signaling pathways, cell cycle control, and stress response mechanisms present in this dinoflagellate species. To date, 1150 *K. brevis* expressed sequence tags (ESTs) have been sequenced. Of these, 36% have high homology to known genes in the GenBank database. Using these sequence data, we have developed probes for known cell cycle regulatory proteins to study the mechanisms controlling the growth phase of bloom formation and for stress proteins involved in adaptation/ survival of *K. brevis* cells as they are exposed to changing water column conditions. Understanding cellular regulation is a prerequisite to developing truly predictive models or species-specific control strategies.

Contact : Fran Van Dolah

Development of a Molecular Probe for *Karenia brevis*

The brevetoxin producing red tide dinoflagellate, *Karenia brevis*, is responsible for closure of shellfish harvesting as well as marine faunal mortalities throughout the Gulf of Mexico and along Florida's Atlantic coast. The serious economic impacts of this harmful algal bloom species have led to the establishment of rigorous monitoring programs for early detection of *K. brevis* and also of research programs aimed at modeling and ultimately forecasting bloom events. For both monitoring and research applications, enumeration of *K. brevis* cells by conventional microscopy is required but is time-consuming and can be problematic when morphologically similar species occur in the same water sample. We have developed a species-specific, fluorescently labeled probe targeting *K. brevis* ribosomal RNA that "lights up" *K. brevis* cells under the epifluorescence microscope and clearly distinguishes them from cells of co-occurring species. Application of this molecular probe will result in enhanced speed and accuracy of microscope-based counting of *K. brevis* cells. Future use in conjunction with advanced instrumentation such as flow cytometers and in-water sensors will establish this probe as a valuable tool facilitating the automated detection of *K. brevis* for a variety monitoring and research applications. *Contact: Greg Doucette*

Identification of an Algicidal Compound from the Cyanobacteria, *Trichodesmium*

Blooms of the brevetoxin producing dinoflagellate, *Karenia brevis,* occurring in the eastern Gulf of Mexico are frequently preceded by blooms of the cyanobacterium, *Trichodesmium erythraeum*. However, in the western Gulf of Mexico, populations of the closely related *T. theibautii* appear to inhibit *K. brevis* bloom formation. Cell extracts obtained from these two *Trichodesmium* species were tested for growth effects against a diverse array of algal groups, including diatoms, dinoflagellates, green algae, and coccolithophores. Algicidal (i.e., algae killing) activity was observed for extracts of *T. theibautii,* while this activity was absent from *T. erythraeum* extracts. Our current research is aimed at determining the chemical structure of this algicidal compound and developing detection methods for use with field samples. Since the presence of *Trichodesmium* and its algicidal compound may regulate initiation of certain *K. brevis* blooms, our ability measure levels of the algicide in natural populations may aid in assessing the potential for bloom formation. *Contact: Steve Morton*

Transfer of Receptor Assay Technology to SW African Countries Initiated

The southwest African countries of South Africa, Namibia, and Angola have either historical or recently emerging problems with one or more groups of marine algal toxins. These countries have requested assistance through the U.N. International Atomic Energy Agency (IAEA) in establishing capabilities for receptor assay-based detection of algal toxins in seafood products. A project planning meeting was held at IAEA Headquarters in Vienna, Austria to develop a regional technical cooperation proposal for the transfer of the Marine Biotoxins Program's receptor assay technology to each of these three African countries. This project will be modeled after an ongoing IAEA-sponsored program in SE Asia, with the African end-users visiting the CCEHBR laboratory next year for training and returning to their home institutions to begin conducting the assays. An inter-calibration study coordinated through our Program will follow, and then receptor assays will be implemented as a component of their respective toxin monitoring programs, which are either well-established (S. Africa) or currently being developed. Acquisition of receptor-based technology will be of immediate benefit to each of our African partners, given their rapidly growing fishery and aquaculture industries along with the accompanying demands for biotoxin testing of products for export to world markets. *Contact: Fran Van Dolah*

New Radioimmunoassay Provides Sensitivity Required to Biomonitor Blood Brevetoxin

The need for definitive toxin identification associated with harmful algal blooms is increasing. However, the relationship between exposure to toxins in the environment and adverse effects is poorly defined. Biomonitoring of toxins in human or animal tissue using blood collection cards provides an efficient means to identify toxins in living animals in order to assess exposure. Collaborative research between CCEHBR and AgResearch Ltd. (New Zealand) has resulted in a new radioimmunoassay for brevetoxins. The radioimmunoassay provided sensitive detection with minimal interference of residual matrix following extraction of blood samples from the cards. The method has been tested in laboratory mice after acute, long term, and low dose exposure to brevetoxins and was determined to detect blood brevetoxin at doses ten times below the lowest observable effect dose and at times up to two days after exposure. Analysis of toxins extracted from blood collection cards using this radioimmunoassay will permit biomonitoring of blood brevetoxin during HAB events and can be anticipated to provide early indications of toxic events and to enhance our ability to predict the toxic consequences of red tides in protected species. *Contact: John Ramsdell*

Dietary Cholestyramine Prevents Brevetoxin Symptoms

There is a need to develop prophylactic treatment to alleviate the adverse effects of dietary exposure to marine algal toxins. Dietary treatment with cholestyramine is one possible means of either binding or disrupting enterohepatic circulation of toxins and thereby mitigating toxic symptoms. While cholestyramine has been used successfully to block the toxic effects of mycotoxins and other toxicants, there is little supportive experimental evidence with algal toxins. Work in collaboration with the U.S. EPA laboratory in Research Triangle Park, incorporated cholestyramine into the diet of laboratory mice, which were then exposed orally to brevetoxin. A 5% (w/w) diet of cholestyramine for one week prior to toxin exposure eliminated the characteristic hypothermic response to a nonlethal dose of brevetoxin. Mice fed the cholestyramine diet displayed no observable symptoms to oral brevetoxin exposure, even when given a higher dose of brevetoxin that caused 50% lethality in one hour in the mice fed the control diet. These exciting results provide the first experimental data that support the effectiveness of this commonly used cholesterol lowering drug to mitigate the adverse of effects of brevetoxin. *Contact: John Ramsdell*

Volunteers Monitor Harmful Phytoplankton Along South Carolina's Coast

The South Carolina Phytoplankton Monitoring Network (SCPMN) began its second year of existence with over 34 groups monitoring state coastal waters for potentially harmful algal species. A total of over 50 sampling sites from all coastal counties of South Carolina are monitored each week. Volunteer groups are composed of both middle and high school students, state park personnel, and citizen environmental groups. This NOAA sponsored community program serves to increase the awareness of constituent groups about the many issues related to harmful algae and directly involves volunteers in coastal stewardship. In the SCPMN's first year of existence, volunteers observed three potentially toxic algae, including *Pseudo-nitzschia*, *Dinophysis*, and *Prorocentrum lima*. Observation and identification of phytoplankton along the South Carolina coast will be useful in developing a species list and record of distribution, as well as alerting NOAA scientists to the presence of potentially harmful species at the many sampling sites. *Contact: Steve Morton*

Confirmation of PSP in the Indian River Lagoon: A New Public Health Issue in Florida

Between 1 January – 25 April 2002, 14 pufferfish poisoning incidents in Florida were reported from the Indian River Lagoon. (IRL), Florida. In collaboration with the Florida Marine Research Institute (FMRI), the U.S. FDA, and NRC Canada, we have confirmed the presence of saxitoxins (STX) in pufferfish in the IRL. This is the first toxic event in Florida waters in which STX has been identified. Concentrations of up to 6238 ìg STX eq/ 100 g were detected in skin, mucus, muscle, and viscera of southern pufferfish, with highest levels in the skin and mucus. STX was also confirmed in conch (*Melongena corona*) and cockle (*Americardia media*) from the IRL, with traces detected in hard clams (*Mercenaria* spp.). Both unialgal cultures of the dinoflagellate *Pyrodinium bahamense* and natural bloom samples (> 3 million cells/L) obtained during fish kills in the IRL tested positive for STX. *Pyrodinium bahamense* var. *bahamense*, the variety found in Florida, has never before been reported to be toxic. Current studies at FMRI are aimed at confirming the origin of the toxic blooms present in the IRL. Recently, the northern IRL has experienced a number of unusual events, including dolphin, manatee, fish, and horseshoe crab mortalities, increased tumor incidence in hard clams, "spicy clams," and reduced natural recruitment and hatchery losses of hard clams. To what extent these events are linked to the emerging issue of toxic *P. bahamense* blooms remains undetermined. Public health risks associated with PSP have resulted in the implementation of new management strategies by the state of Florida, which previously has had to regulate shellfish harvests only for brevetoxins. *Contact: Fran Van Dolah*

2003: Sequencing of 8000 Expressed Genes in *Karenia brevis* Provides Foundation for Development of DNA Microarrays

Karenia brevis is a brevetoxin producing dinoflagellate responsible for red tides in the Gulf of Mexico, where it causes extensive fish kills, marine mammal mortalities, and human illness. Significant progress has been made towards understanding the factors controlling *K. brevis* blooms at the oceanographic level. However, little is known about the molecular and cellular controls that mediate responses of *K. brevis* to environmental cues leading to bloom formation and bloom termination. To facilitate research into the molecular biology of *K. brevis*, we embarked on a functional genomics project in FY01 with the development of a cDNA library as a gene discovery tool for identifying regulatory pathways present in this primitive eukaryote. This year, sequencing of ~8000 expressed sequence tags (ESTs) for genes expressed in the library has been completed and the sequences compared to the non-redundant GenBank sequence database using the basic local alignment search tool (BLAST). Oligonucleotide probes unique to each of the ESTs identified in the library are currently being designed for application to microarrays that will be developed in the next year. The DNA microarrays will be utilized to identify genetic pathways involved in bloom growth and toxicity. *Contact: Fran VanDolah*

Identification of Stress Proteins in *Karenia brevis* May Provide Insight into Mechanisms of Bloom Termination

Blooms of *Karenia brevis* initiate offshore and become a threat to coastal ecosystems and humans only when transported inshore by prevailing wind and oceanographic conditions. Once in coastal waters, *K. brevis* cells experience dramatic changes in their environment, resulting in temperature, salinity, light, turbulence, and oxidative stresses. The longevity of coastal *K. brevis* blooms depends on the degree to which they can adapt to environmental stresses encountered in coastal waters. Thus, understanding the physiological basis for adaptation (or failure to adapt) to the coastal environment is a prerequisite to understanding the mechanisms leading to bloom termination. Studies completed in FY03 identified for the first time a suite of stress proteins expressed in *K. brevis* and characterized their responses to heat, light, and oxidative stresses. This information will serve as the foundation for investigating mechanisms leading to dinoflagellate cell death and bloom termination.

Contact: Fran VanDolah

Production of Brevetoxin Brought to High Analytical Purity Provides Component Necessary for Toxin Detection Methods

Analytically pure toxin standards are a critical component for toxin detection methods. Several methods commonly used to detect brevetoxins in shellfish and marine mammals require a radio-labeled form of brevetoxin. To produce this essential compound, brevetoxin must first be extracted from large batches of laboratory cultivated algae (approximately 400 liters of *Karenia brevis*). The demand for bulk purified toxin has lead to the development of a two stage toxin production method using preparative liquid chromatography-mass spectrometry. Fractions that meet the desired mass, chromophoric, and retention time characteristics are further subjected to nuclear magnetic resonance spectroscopy for purity analysis. This final quality assurance step ensures that contaminants not amenable to ionization are completely removed and provides a much higher degree of accuracy in developing toxin reference and standard materials. The final product for radiolabeling will be sufficient to meet toxin detection needs of the research community for the next several years. *Contact: Steve Morton/Peter Moeller*

Collaboration with Naval Research Laboratory Successfully Tests Portable Biosensor for Toxic Algae

On-line, near-real time detection systems for harmful algal species and their toxins is a rapidly emerging field aimed at forecasting bloom development, persistence, and toxicity as well as providing data to facilitate rapid and more effective responses to harmful algal blooms. Recently the Naval Research Laboratory demonstrated that cultured neuronal networks grown over microelectrode arrays (MEAs) are capable of detecting brevetoxins, saxitoxin, and domoic acid. An on-site collaboration at the Marine Biotoxins Program using a prototype portable battery-operated unit containing a central core of living neurons growing on a biosensor chip sought to determine if the sensor could detect these toxins directly in the seawater growth medium of *Alexandrium fundyense* and *Karenia brevis* The instrument responded with positive toxin signatures from the sonicated medium of each red tide alga, but not from non-toxic isolates of the same algal genus. This successful trial provided evidence that the prototype MEA has the capacity to detect toxins associated with cells of toxic algal species and exhibits the potential for monitoring toxin levels during harmful algal blooms. *Contact: John Ramsdell*